

Effects of pesticides on photosynthesis

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I. Introduction

With the advent of insecticides,¹ fungicides,¹ and antibiotics¹ on a large scale, the damages caused to crops could be mitigated but the interference of these chemicals with various metabolic pathways of plants has seldom been studied in detail as little time is available to scientists engaged in research to develop new "pesticides" at such a pace as the needs of farmers demand today. "Pesticides" in a broader sense include a wide range of chemicals such as insecticides, fungicides, rodenticides, herbicides, miticides, and antibiotics (Nayar *et al.* 1979). In their attempt to secure food, insects inflict serious damages to almost every part of plants and the seriousness of the attack may be decided not only by the nature of the injuries inflicted but also by the susceptibility of the plants concerned to insect attack. The two main types of insect feeding—the biting and

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¹Chemicals mentioned in the text are identified in Table I.

Table I. Chemical designations of compounds mentioned in text.

Designation	Identity
DCMU	3-(3',4'-dichlorophenyl)-1,1-dimethyl urea
DBMIB	2,5-dibromo-3-methyl-6-isopropyl- <i>p</i> -benzoquinone
DNP-INT	2,4-dinitrophenyl ether of 2-iodo-4-nitrothymol
DNP-BNT	2,4-dinitrophenyl ether of 2-bromo-4-nitrothymol
DINOSEB	2- <i>sec</i> -butyl-4,6-dinitrophenol
DN- <i>O</i> -C	4,6-dinitro- <i>o</i> -Cresol
DDT	4,4-dichlorodiphenyltrichloroethane
Trifluralin	<i>N</i> -di-isopropyl-dinitro-trifluroromethyl-aniline-trifluralin
Methoxychlor	1,1-(2,2,2-trichloroethylenedene)-bis-(4-ethyl methoxy benzene)
Methyl-parathion	<i>O,O</i> -dimethyl <i>O-p</i> -nitrophenyl phosphorothioate
Parathion	<i>O,O</i> -dimethyl-4-nitrophenyl phosphorothioate
Nitrofen	2,4-dichlorophenyl- <i>p</i> -nitrophenyl ether
Atrazine	2-chloro-4-ethyl-amino-6-isopropylamino- <i>s</i> -triazine
Filipin	A polyene antibiotic produced by <i>Streptomyces filipinensis</i>
Amphotericin B	A polyene antibiotic
Antimycin A	A macrolactone antibiotic produced by <i>Streptomyces</i> species
Valinomycin	Antibiotic produced by <i>Streptomyces fulvissimus</i>

sucking types—are well known, and the effects of feeding may be direct/causing outright injuries or indirect through helping in the transmission of diseases—bacterial, fungal, or viral (Nayar *et al.* 1979). In the process of insect feeding, venom-like substances such as melittin (Bishop and Kenrick 1980), a polypeptide component of bee-venom, are released into the conducting tissues of plants and are found to hamper the metabolic pathways. Melittin is reported by Bishop and Kenrick (1980) as an inhibitor of photosynthetic activities and its mechanism of action in detail is dealt with in the following pages. Therefore, pesticide application, either systemic or foliar, in agriculture is important and imperative as it offers early and brisk control of pests or diseases that damage agriculturally important crops at all stages of growth. But this does not endorse the fact that the yield of the crop is ultimately good and rich in nutrition.

Quantitative rise in the yield of crops due to pest control certainly cannot rule out that the application of several kinds of chemicals in the pest control or disease control has not affected the important metabolic pathways such as photosynthesis and respiration (Bajaj and Ghosh 1975). Since photosynthesis is a food-processing mechanism both for plants and people, significance has been attached to effects of these chemicals on photosynthesis. Predictably Sances *et al.* (1981) reported recently that pesticides had reduced the yield of lettuce crop, cultivated both at South Coast Field Station, Santa Ana, and Citrus Research Station, Riverside (U.S.A). (The discussion about the results of these experiments is furnished in the following pages.) Further, the quality of food stored in fruits

and seeds due to photosynthesis, which is edible for living beings, is by and large dependent on any deviations that take place in the mechanism of photosynthesis, but little work has been done on seed content analysis at different stages of development of crops, while a number of both hazardous as well as otherwise chemicals have been under constant use.

Photosynthesis is a delicate mechanism and is easily susceptible to a number of fluctuations or deviations which could be due to any change both in the environment and soil. Cations like Na^+ , K^+ , Mg^{+2} , Cu^{+2} (Spencer and Possingham 1960, Jagendorf and Smith 1962, Fredericks and Jagendorf 1964, Spencer and Unt 1965, Izawa and Good, 1966 a, 1966 b, Gross and Packer 1967, Gross *et al.* 1969, Murakami and Packer 1971, Habermann 1969, Gross *et al.* 1970, Gross and Libby 1972, Cedeno-Maldonado and Swader 1972, Buchaner 1973, Gross and Hess 1973, Mohanty *et al.* 1973, Gross and Prasher 1974, Gross and Hess 1974, Vandermeulen and Govindjee 1974, Gross *et al.* 1976, Smillie *et al.* 1976, Davis *et al.* 1976, Vierke and Struckmeier 1977 and 1978, Bose and Arntzen 1978, Shioi *et al.* 1978 a, 1978 b, Govindjee *et al.* 1979, Bohner *et al.* 1980, Goran and Oquist 1980), Ca^{+2} (Gross and Hess 1974, Barr *et al.* 1980, Troxel *et al.* 1980), Pb^{+2} , Cd^{+2} (Buchaner 1973, Bazzaz and Govindjee 1974 a, 1974 b, Li and Miles 1975, Wong and Govindjee, 1976), Hg^{+2} (Trebst 1963, Kimimura and Katoh 1972, Katoh 1972, Honeycutt and Krogmann 1972, Ouitrakul and Izawa 1973, Yocum and Guikema 1977), and Zn^{+2} (Hewitt 1958, Buchaner 1973, Agrawala 1977, Davies 1979, Tripathy and Mohanty 1980, Shrotri *et al.* 1981), were found to interfere with photosynthetic mechanism at various points of electron transport and also at the site of ATP generation. Particularly these cations accumulate in the environment surrounding plants due to extensive application of pesticides, due to smelters and mines (Buchaner 1973), and due to several ecological factors. There can be several other reasons, also, for the accumulation of these cations in toxic proportions such as low saline and high saline soil conditions, etc., that have a greater synergistic (added effect by pesticides application) impact on photosynthesis.

Inorganic pesticides like borax ($\text{Na}_2\text{B}_4\text{O}_7$), copper acetoarsenate (CH_3COO_2) $\text{Cu} \cdot 3\text{Cu}(\text{AsO}_2)_2$, potassium antimonyl tartarate, Bordeaux mixture (Cu-Ca complex), sodium selenate (Na_2SeO_4), wettable sulphur (S_2), and zinc phosphide (Zn_3P_2) and zinc sulphate (ZnSO_4) are under extensive application in pest control. There may be several other pesticides which contain one or the other cations mentioned above.

Similarly organic pesticides include dinitrophenols, dinitrophenyl ethers, diphenylethers, pyrones, alkyl substituted derivatives of halogenated *p*-benzoquinones, and triazinones. In addition, antibiotics like Dio-9 (McCarty *et al.* 1965), chloramphenicol and cycloheximide (Nielsen 1975), amphotericin B (Nolan and Bishop 1975 and 1978), antimycin A (Tagawa *et al.* 1963, Izawa *et al.* 1967, Trebst *et al.* 1970, Bohme *et al.* 1971) and valinomycin (Telfer and Barber 1974, Voegeli *et al.* 1977) have been in use in the horticulture to arrest certain pests on vegetable crops.



Fig. 1. Washed isolated chloroplasts of *Vicia faba* with outer membranes and stroma lost. Granal stacks are clearly seen as dark squares, hither and thither; X 4,800 (courtesy of R. Leech and A. D. Greenwood).

II. Role of cations and salts

a) Monovalent and divalent cations

The role of both monovalent and divalent cations has been studied to some extent in photosynthesis keeping in view their nutritional value, toxic concentration ranges involved, and the stress of the low saline and high saline conditions of soil on plants.

These cations are supplemented to plants both from soil as well as environment and by way of adding manures and fertilizers. Cations are required for Hill reaction activity and to keep the granal stacks intact when the outer envelope of chloroplasts is broken (Figs. 1 and 2). However, the integrity of chloroplasts would be lost due to mineral deficiencies also, as reported by Spencer and Possingham (1960) in tomato and Hall *et al.* (1972) in maize. Smillie *et al.* (1976) concluded that particularly cations (Na^+ , K^+) in high concentrations, 100 to 200 mM NaCl or KCl, have a great effect on the structure and activity of the chloroplasts. Below 8 mM NaCl concentration the thylakoids swell and there was a gradual loss of Hill activity and the capacity to maintain a light-dependent proton gradient. Maximum activation of photoreduction of ferricyanide occurred at 30 mM NaCl and divalent cations like Mg^{+2} , Ca^{+2} , Mn^{+2} are 12 times more effective than monovalent cations. Also divalent cations are more than 200

times as effective as monovalent cations in arresting the loss of Hill activity and fall of light-dependent proton gradient. However, the role of cations in photoreductive reactions is not yet clear. Gross and co-workers (1969) reported inhibition of ferricyanide reduction by cations. Spencer and Unt (1965) noticed considerable increase in the rate of photoreduction of ferricyanide or trichlorophenol (TCPIP) with the gradual removal of cations and as a result noncyclic and cyclic photophosphorylations were lost. While removal of cations can cause an increase in the photoreductions of ferricyanide, there is evidence elsewhere (Shavit and Avron 1967) to show that cations stimulate ferricyanide photoreductions. These are a few contradictions which, of course, warrant further investigation. Jagendorf and Smith (1962) and Spencer and Unt (1965) observed that removal of cations from chloroplasts resulted in loss of phosphorylative activity and this decrease in activity was prevented by monovalent and divalent cations. They have also found that the loss of phosphorylative activity is followed by the rise in the rate of photoreduction of ferricyanide at low salt concentrations. It may also be noted that ability of chloroplasts to retain a light-dependent proton gradient would be reduced at low salt concentrations.

Similarly, cations can affect many other phenomena. The role of cations in the excitation energy distribution, termed "Spill Over", between two photosystems is one of the most important among them (Mohanty *et al.* 1973, Gross and Hess 1974, Vandermeulen and Govindjee 1974, Gross and Prasher 1974,

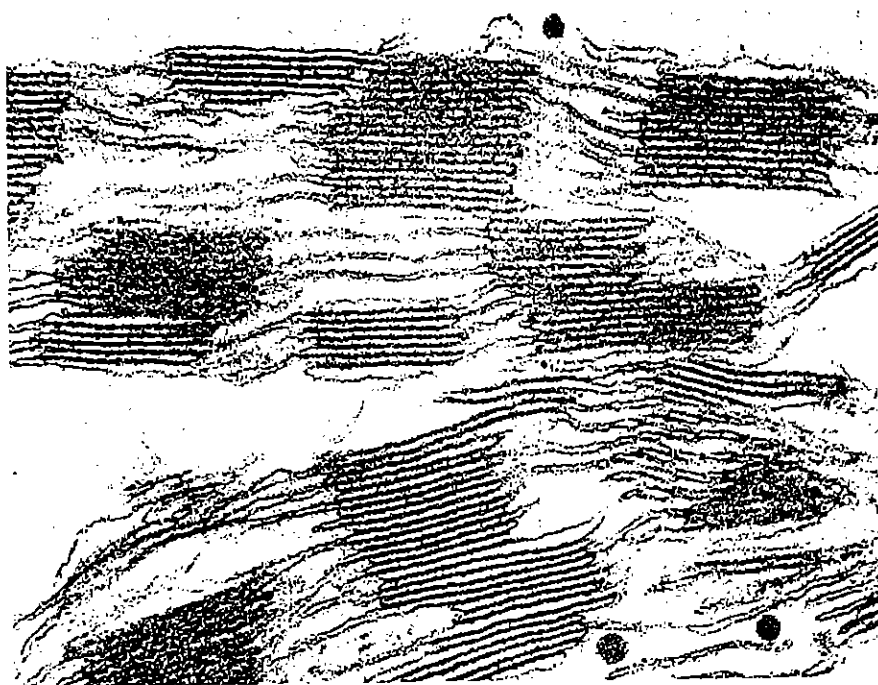


Fig. 2. Naked chloroplast lamellar systems with tightly stacked grana and an intrathylakoid space about 15mm; X 64,000 (courtesy of Niels C. Nielsen, Purdue University, West Lafayette, Purdue, IN, U.S.A).

Gross *et al.* 1976, Davis *et al.* 1976). Divalent cations and monovalent cations regulate excitation energy distribution between two photosystems. Divalent cations, particularly, are known for their decreasing effect of "Spill Over" of excitation energy from Photosystem II (PSII) to Photosystem I (PSI). The regulation of excitation energy distribution is of significance to the optimization of *in vivo* in photosynthesis at low light intensities. The higher activity of PSII in the presence of low concentrations of divalent cations is suggested to be due to reduced energy transfer from PSII to PSI. Govindjee *et al.* (1979) concluded that Mg^{+2} enhances the light-limiting electron transport rate through PSII in the pH range 5.4 to 8.2 and decreases that through PSI at pH 7.1 and 8.2. The percent of increase in PSII, however, is about twice the percent of decrease in PSI. Also there are several contradictory reports earlier as to the role of Mg^{+2} in the excitation energy distribution between two photosystems. Some authors even suggested that Mg^{+2} (Bose and Arntzen 1978) may activate reaction centre of PSII which, however, was later contradicted by Govindjee *et al.* (1979).

Malakondaiah and Rao (1979 and 1980) reported that under high saline conditions K^+ and Ca^{+2} contents have been lowered and Na^+ accumulated in the plants resulting in the reduction of plant growth and photosynthesis activity. They showed that addition of phosphor (P_4) decreased the Na^+ content and increased the photosynthesis activity.

Besides, the effect of calcium (Ca^{+2}) on photochemical reactions has been studied from the point of view of its role in excitation energy transfer between two photosystems (PSII and PSI). Gross *et al.* (1976) reported that Ca^{+2} ions in the range of 1 to 10mM concentration decreased the quantum yield of PSII and increased that of PSI. Fredericks and Jagendorf (1964) found that Ca^{+2} salts stimulated, but Na^+ and K^+ salts inhibited Hill reaction rates in *Anacystis nidulans*. Studies of DCMU-insensitive silicomolybdate reduction by PSII confirmed that moderate concentrations of $CaCl_2$ (20mM) stimulated Hill reaction at pH 0.6 and pH 0.8, while Mg^{+2} , Sr^{+2} and Ba^{+2} ions inhibited Hill reactions at similar concentrations (Barr *et al.* 1980). Troxel *et al.* (1980) attempted to describe the role of Ca^{+2} by prior elimination of Ca^{+2} from chloroplasts, using EGTA, a calcium chelator (EGTA = ethylene glycol bis (- aminoethyl ether) -N,N-tetracetic acid). They found that, from EGTA chelator studies, Ca^{+2} has two sites of action, as reported earlier by Gross and Hess (1974), and also pointed out that the site II of calcium function is associated with PSII reaction center and the site I of calcium function is located close to Plastocyanin (PC). They opined that Ca^{+2} organizes the way, in which PC attaches itself to the chloroplast membranes, to make it function. However, the actual mechanism of Ca^{+2} is yet unknown though Gross and his associates (1974) and Davis *et al.* (1976) emphasized its participation as a nonspecific divalent cation in the distribution of excitation energy, between two photosystems, termed "Spill Over".

The influence of copper, lead, and cadmium ions on the electron flow and PSII in particular have also been investigated in view of the ecological implica-

tions. Copper has long been known to inhibit photosynthesis and it is an essential micronutrient (Sommer 1945). Vierke and Struckmeier (1977 and 1978) reported that the binding of $50\mu\text{M}$ Cu^{+2} to proteins of the thylakoid membrane would result in bringing about some conformational changes affecting the PSII activity. Cedeno-Maldonado and Swader (1972) have shown that Cu^{+2} inhibition of isolated spinach chloroplasts increases with a decreasing $\text{Chl}/\text{Cu}^{+2}$ ratio and that Cu^{+2} preferentially binds to inhibitory sites in light. Habermann (1969) documented that Mn^{+2} effectively minimizes the sensitivity of Cu^{+2} inhibition and that Cu^{+2} site of action is accepted as located generally on the oxidizing side of PSII.

Recently, Bohner *et al.* (1980) and Goran and Oquist (1980) had separately studied the effects of Cu^{+2} ions on photosynthetic electron flow. Bohner *et al.* (1980) documented that the concentration of PC and Cyt c-553 are inversely proportional to the concentration of cupric ions. However, they noticed no significant change in the electron flow at a concentration of copper below $10\mu\text{M}$. At higher concentration Cu^{+2} has two sites of inhibition (Goran and Oquist 1980): one on the oxidizing side of PSII and the other on the reducing side of PSI. At exceedingly higher concentrations, $40\mu\text{M}$ in the case of *Scenedesmus* and 1mM in respect of *Chlorella* (Gross *et al.* 1970), the destruction of photosynthetic apparatus also took place (Bohner *et al.* 1980). Even at low concentrations of $2\mu\text{M}$, Cu^{+2} inactivated ferredoxin and the water-splitting reaction. Shioi *et al.* (1978 a and b) showed inhibition of the DCPIP-Hill reaction was affected by Cu^{+2} , with both water and diphenyl carbazide as electron donors, and copper at a concentration of $5 \times 10^{-6}\text{M}$ inhibited the activity of PSII ($\text{H}_2\text{O} \rightarrow \text{DCPIP}$) by 50% in spinach. According to them when the photophosphorylation was previously uncoupled by $6.6 \times 10^{-4}\text{M}$ NH_4Cl , the low Cu^{+2} concentration of 10^{-6}M also became inhibitory. But in the absence of previous uncoupling by NH_4Cl , Cu^{+2} at 10^{-6}M concentration could act as uncoupler (stimulator of electron flow) also in coupled chloroplasts until the concentration of Cu^{+2} reached inhibitory level.

Likewise 1mM Pb^{+2} (Buchaner 1973, Bazzaz and Govindjee 1974a, Wong and Govindjee 1976) salts affect photosystem II activities; inhibition or stimulation depending on pH. Cadmium (Buchaner 1973, Bazzaz and Govindjee 1974 b, Li and Miles 1975) salts do affect the donor side of PSII in the range of 1 to 10mM concentrations.

Recently Tripathy and Mohanty (1980) reported firstly on the nature of inhibition of photosynthetic electron flow by zinc (Zn^{+2}) in barley. Earlier Agrawala *et al.* (1977) had shown that zinc inhibited the growth and dry matter production in barley. It was reported by Tripathy and Mohanty (1980) that Zn^{+2} at a lower concentration of 0.5 to 1mM had partly inhibited the methyl viologen photoreduction associated with oxygen uptake and at higher concentration of 3mM , it (zinc) completely inhibited the methyl viologen associated oxygen uptake. They concluded that zinc toxicity appears to interfere with the primary photoreduction and electron transport activities of chloroplasts. Further

more, Shrotri *et al.* (1981) reported that the mode of action of zinc in zinc-deficient maize leaves (*Zea mays*) is different from that of others as maize belongs to C₄-group of photosynthetic plants. They also observed that exogenous addition of ZnSO₄ to zinc-deficient leaves does not restore the electron transport, photophosphorylation, and ¹⁴CO₂ fixation. On the contrary it inhibits these photochemical activities as observed earlier by Tripathy and Mohanty (1980). According to Shrotri *et al.* (1981) the only way out to overcome the effects of zinc deficiency is exogenous addition of co-factors such as NADPH, ATP, and PEP (phospho-enol-pyruvate) which could restore electron transport, photophosphorylation, and ¹⁴CO₂ fixation instead of addition of Zn.

b) Salt-induced changes

The effects of several salts on the conformation and function of chloroplast membranes have been studied by Izawa and Good (1966 a and b), Gross and Packer (1967), Gross (1971), Mohanty *et al.* (1974), and Barr and Crane (1975). A most striking structural change in chloroplasts first observed by Izawa and Good (1966 a and b) was disintegration of grana in chloroplasts isolated in high salt medium and then exposed to a low salt concentration. The other changes indicative of serious effects of high salt conditions were the volume of the chloroplast lamellar systems was increased (Gross and Packer 1967) and their light scattering properties were decreased. Barr and Crane (1975) reported about the chaotropic action of salts like KNO₃, NaSCN, KCl, NaCl, and K₂SO₄, and Barr and Crane described chaotropic effect as solubilization of proteins and oxidation of lipids of membranes by these salts. Effect of these "chaotropes" on partial reactions of photosynthesis and the cation response to these chaotropic salts have been elucidated by Barr and Crane (1975). Whereas KNO₃, NaCl, KCl, and MgCl₂ inhibited the PSI reaction (Ascorbate + TMPD → methyl viologen) rate at 1M concentration, K₂SO₄ nonchaotropically stimulated the PSI reaction and almost doubled the PSI reaction rate. At low concentrations (10mM to 200mM) most of the salts except NaSCN stimulated electron transport, while 25 to 80% inhibition of PSI was noticed at higher concentrations. The silicomolybdc acid reduction (diphenyl carbazide as donor to PSII) by PSII was inhibited by low concentrations (0.1M) of I⁻ and SCN⁻, but Br⁻ had less effect even in high concentrations (1M). However, K₂SO₄ recovered from the inhibition at higher concentrations. The diphenyl carbazide supported dichlorophenol indophenol (DCPIP) reduction by PSII was inhibited by low concentration of NaCl. All other chaotropic agents used inhibited reaction rates at low and high concentrations according to an increased degree of severity; NaCl > KCl > MgCl₂ > KBr > KNO₃ > KI > NaSCN.

The chaotropic agents Cl⁻, NO₃⁻, and SO₄⁻² inhibited cyclic and noncyclic photophosphorylations, also. However, low concentrations of MgCl₂ had become necessary for more than 50% inhibition of noncyclic photophosphorylation. This suggests the influence of cation Mg⁺² on the action of Cl⁻. Cyclic photo-

phosphorylation was completely inhibited by the chaotropic action of MgCl_2 only at low concentrations. However, KCl , KNO_3 , and NaCl did not affect the cyclic photophosphorylation.

The inhibitory effects were caused by the release of manganese from its site of action in the chloroplasts. Further, the chaotropic action of the above-discussed salts and other salts on chloroplasts PSII system is a multiple effect including chaotropic interaction between chloroplast proteins and water structure. The inhibition of cyclic and noncyclic photophosphorylations could be due to the interaction of chaotropic agents with coupling factor 1 (CF1). Gross (1971) also observed uncoupling of photophosphorylation and inhibition of proton binding by quaternary ammonium salts and zwitterionic buffers. Mohanty *et al.* (1974) reported salt-induced alterations of the fluorescence yield and of emission spectra in *Chlorella pyrenoidosa*. According to Mohanty *et al.* (1974) salts (KCl , MgCl_2 , K-acetate, Na-acetate, NH_4 -acetate) inducing changes are not directly related to redox reactions of photosynthesis, but these salts could cause a decrease in the mutual distance between two photosystems by changing the microstructure of chloroplasts *in vivo* as a result of which the "Spill Over" of excitation energy from strongly fluorescing system II to weakly fluorescing system I is easily facilitated. They observed further that the low concentrations of (0.1M) Na-acetate and K-acetate had an ionic effect, whereas the high concentrations of MgCl_2 (0.4M) had an osmotic effect on the fluorescence and spill over of excitation energy distribution.

Organomercuric compounds like phenyl mercuric acetate (Honeycutt and Krogmann 1972) and inorganic mercury compounds like calomel (HgCl_2) were found to be inhibitors of photosynthesis (Kimimura and Katoh 1972). Phenyl mercuric acetate inhibits the ferredoxin-NADP reductase (Honeycutt and Krogmann 1972) and HgCl_2 inhibits at the site of plastocyanin (PC) in the downhill electron carriers (Fig. 3). Kimimura and Katoh (1972) and Katoh (1972) described the mode of inhibition of photosynthetic electron flow by HgCl_2 . It directly reacts with PC. HgCl_2 affects coupling also (Trebst 1963). Trebst (1963) reported that at concentration of below $1\mu\text{M}$, HgCl_2 couples the photosystem II electron flow. Incidentally, KCN also inhibits the electron flow at plastocyanin site at concentrations equal to 10mM and higher than that. Ouitrakul and Izawa (1973) and Yocum and Guikema (1977) studied these in detail and they introduced different combinations independently to understand the coupling of PSII and photophosphorylation driven by the PSII.

In short, the mode of action of cations and several salts discussed here on the function and conformation of chloroplasts could be three dimensional as outlined below: (1) when the electron flow from PSII to PSI is blocked, there will be a steep rise in the level of fluorescence which implies that the interconvertible light energy is re-emitted out unutilized by the plant. Conversely, if the inhibition of electron transport occurs on the PSII donor side, the fluorescence level would decrease sharply. However, the effects of the cations and salts on the elec-

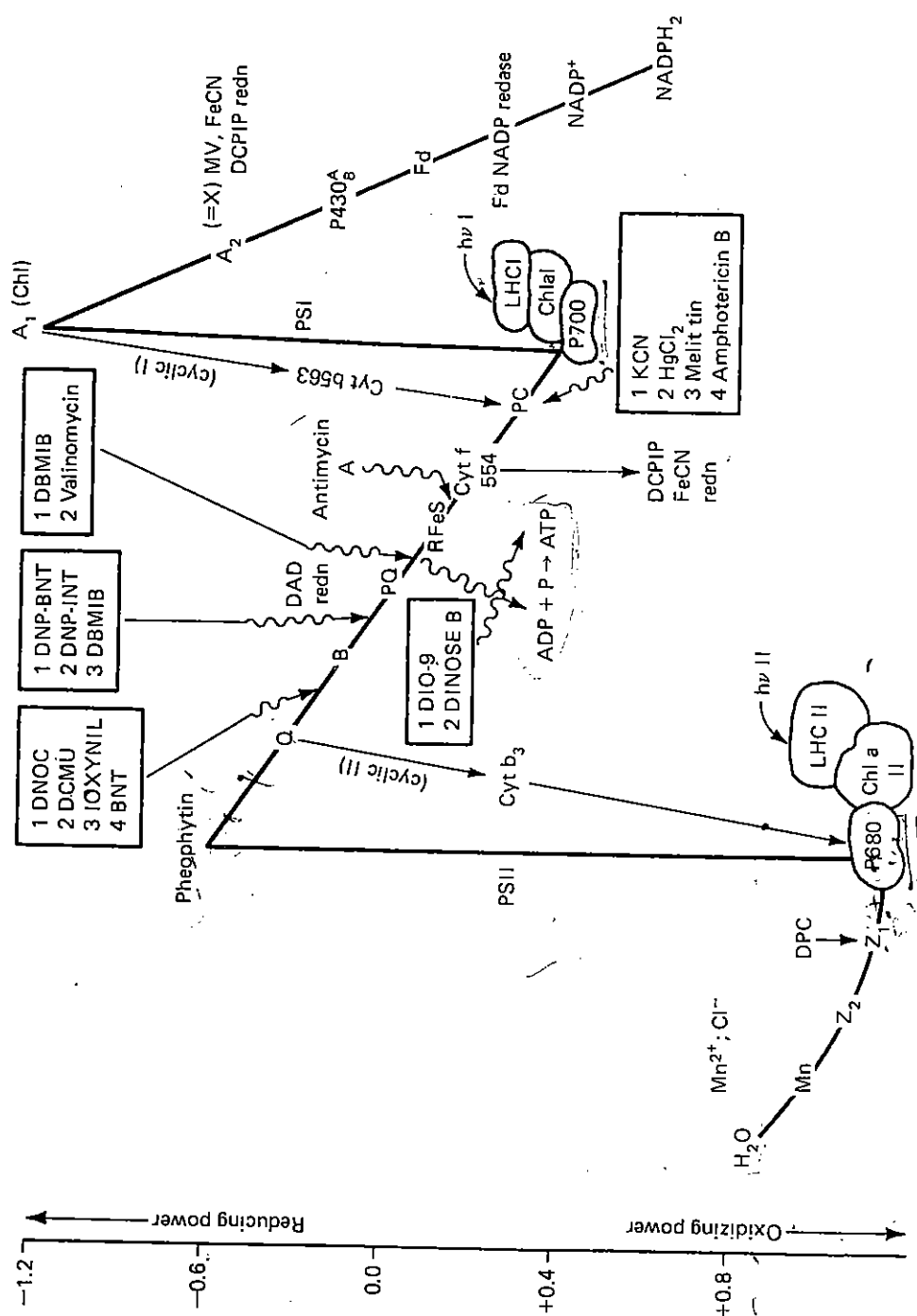


Fig. 3. There are two photosystems PSII and PSI which consist of independent reaction centers, P680 and P700 (680 and 700 stand for their difference of absorption in the region of visible spectrum). Each reaction center is supported by a light-harvesting complex (LHC) constituting a number of accessory pigments (carotenoids and phycobilins) including chlorophyll *a* and *b* molecules. Since each system has an antenna of pigments separately, both PSII and PSI would induct electrons into system soon after their antennae are hit by photons ($H\nu$). Each photon imparts energy to the reaction center. Since two molecules of water split and release 4 electrons into system, total eight quanta are necessary for the liberation of an oxygen molecule (1 electron = 2 quanta). Each photosystem has a primary electron acceptor and a donor. PSII has 'Q' (a quinone molecule or quencher of fluorescence) as the primary electron acceptor, and 'Z₂' as primary electron donor. Similarly PSI has A₁ (chlorophyll) as electron acceptor and plastocyanin (PC) as the primary electron donor. Remaining electron carriers B (a protein), PQ (plastoquinone), RFeS (Rieske Fes Centres), Cyt f 554 (cytochrome f with 554 nm of absorption), PC (plastocyanin) are the constituents of down-Hill electron chain. A site of ATP (adenosine triphosphate) generation is located between PQ and Cyt f. When both P 680 and P 700 are excited by light, each would eject an electron into system and draw an electron from the corresponding primary electron donor. P 680 draws electron from 'Z₂', which in turn oxidizes water. P 700 draws electron from plastocyanin. The electrons released into system would pass through several electron carriers and the electron flow is coupled to ATP formation which is called photophosphorylation. There are two known photophosphorylations: (1) cyclic (both cyclic I and cyclic II) and (2) noncyclic. Totally two ATP molecules would be produced in the process. As a result of photophosphorylations ATP + NADPH would be produced which is utilized by the plant in dark phase for carbon dioxide fixation. ATP + NADPH is together known as reducing power.

Several inhibitors are shown in the figure which exhibit the electron flow. As a result of inhibition ATP synthesis would not take place. As cited in the figure, DBMIB inhibits both before and after the PQ at different concentrations. Melittin, a bee venom, was reported by Bishop and Kenrick (1980) to have inhibited the electron flow at PC site. Some inhibitors act as 'uncouplers' also. When the electron flow is uncoupled from photophosphorylation, no ATP synthesis takes place, but electron flow continues. Similarly some act as 'energy transfer inhibitors' and dissociate ATP into ADP + iP (inorganic phosphate).

tron transport could be noticed on the basis of the difference in the level of fluorescence from the steady state fluorescence (F_0). (2) When the concentration of cations and salts either exceed or fall below the required concentration, conformational changes occur in chloroplasts and the photosynthetic mechanism would be adversely affected, but such conformational changes need not always be severe or drastic. Further, certain membrane-bound electron carriers and enzymes of chloroplasts are released into the inner thylakoid vesicles from their site of action at exceedingly high or toxic concentrations of the cations and salts rendering the photosynthetic mechanism ineffective. (3) When the excitation energy distribution "Spill Over" between two photosystems is improperly regulated by the cations (monovalent and divalent cations) the turn out of photophosphorylation (ATP + NADPH synthesis) would be at low key and the very purpose of photosynthesis in plants is defeated.

There could be several other effects not falling into any of the effects mentioned above, yet of great significance to plant and environment. Owing to innumerable effects these cations exert on chloroplast structure and function, a clear picture of action of a number of salts and chemicals applied in agriculture or horticulture has not yet emerged. It is, thus, imperative to know the effects of these cations, the influence they exert on the chloroplasts, and the critical concentration ranges involved.

It is not the intention of the author to misinterpret the importance of the chemicals containing one or the other cations or salts mentioned above, but he is of the opinion that most of such chemicals warrant a deep study and investigation as the dosages recommended for arresting pests, or diseases (due to bacteria, fungi, and viruses) are far higher and tend to be toxic to the metabolic reactions of the plant in general and to the photosynthesis in particular.

III. Role of functional groups of a few pesticides

It is now certain that plastoquinone (PQ), plastocyanin (PC), and a couple of cytochromes (Cyt b 559 and Cyt f 553) are universally present in all photosynthetic organisms. Further, the fact that the first ATP generation site is located immediately after PQ in the down Hill electron carrier chain has ascribed greater significance to PQ in the research on photosynthesis as the sites closer to PQ are found to be more susceptible to inhibition. By the time this fact was realized, a number of commercially important pesticides had come to stay. The most important among them, which are being researched partly, are mentioned earlier. In fact, the last two decades witnessed remarkable research in this direction and it has been reviewed by several authors taking into account more and more recent findings (Corbett 1974, Moreland and Hilton 1976, Trebst and Draber 1978, Trebst 1980, Murthy 1981).

Indubitably most of the pesticides (particularly herbicides) are either inhibitors of photosynthetic electron flow at the site of the primary quencher (Q) of

PSII and before the reduction of PQ, or inhibitors of electron flow after the functional site of PQ, inhibiting the reoxidation of plastoquinol pool. Ureas like DCMU (diuron) and its analogues (anilides, bis-carbamates, uracils, triazines, triazinones, pyridazinones, pyrimidinones, and trifluorobenzimidazoles), phenols like ioxynil, halogen-substituted nitrophenols like 2-bromo-4-nitrothymol or 2-iodo-4-nitrothymol, and alkyl substituted dinitrophenols like 2-*sec*-butyl 4, 6-dinitro-phenol (dinoseb) and 4,6-dinitro-*o*-cresol inhibit photosynthetic electron flow before the reduction site of PQ (preferably between Q and B). On the other hand, benzoquinones like DBMIB, and diphenylethers of bromonitrothymol and iodonitrothymol, inhibit the electron flow on both sides of PQ at different concentrations (Fig. 3). Thus two distinct classes of inhibitors have originated with different sites of action on either side of PQ. Robinson *et al.* (1977) described a few dinitroanilines which also inhibit photosynthesis. As regards pyrones, Kawamura *et al.* (1980) pointed out that neither of these compounds undergoes any redox reaction nor induces any additional reaction even at concentrations as high as 10^{-3} M. Actually DBMIB and many other types of quinones undergo redox reactions as quinone structure is responsible for it. This quinone structure has been replaced with a form that is inactive in redox reaction in pyrones but their analogous character to benzoquinone remains intact (Fig. 4).

Since several pesticides are found to bind themselves on either side close to Q or PQ (Fig. 3), attempts have been made to identify the receptor proteins for each pesticide. Mullet and Arntzen (1981) successfully identified a 32 to 34 k dalton receptor polypeptide as the triazine herbicide binding site. PSII is in general known for its high sensitivity to herbicide action. Therefore, PSII depleted of 32 to 34 k dalton polypeptide was useful to determine the common herbicide binding site. They found that SPII devoid of 32 to 34 k dalton polypeptide was little sensitive to DCMU but largely sensitive to dinoseb. Hence, Mullet and Arntzen (1981) proposed that a general herbicide binding site (32 to 34 k dalton polypeptide) is common to atrazine, diuron, and dinoseb, and is formed of at least two polypeptides which determine affinity and mediate herbicide-induced inhibition of electron transport on the acceptor side of PSII. One of the significant developments of research on biochemistry of photosynthesis is to induce resistance or insensitivity of DCMU and its analogues into photosynthetic reactions. Towards this end, several mutant resistance strains of *Brassica* and *Amaranthus* were produced (Murthy 1981).

However, a little work has been done on the effects of all categories of pesticides (excepting a few herbicides) on photosynthesis. Sances *et al.* (1981) reported recently that parathion reduces the rate of photosynthesis possibly by inhibiting certain processes in the chloroplast and, as a result, lettuce yield is adversely affected. Sances *et al.* (1981) selected pesticides of several classes such as DDT, methoxychlor, parathion, methyl-parathion, and methomyl and observed that all the pesticides except methoxychlor had affected photosyn-

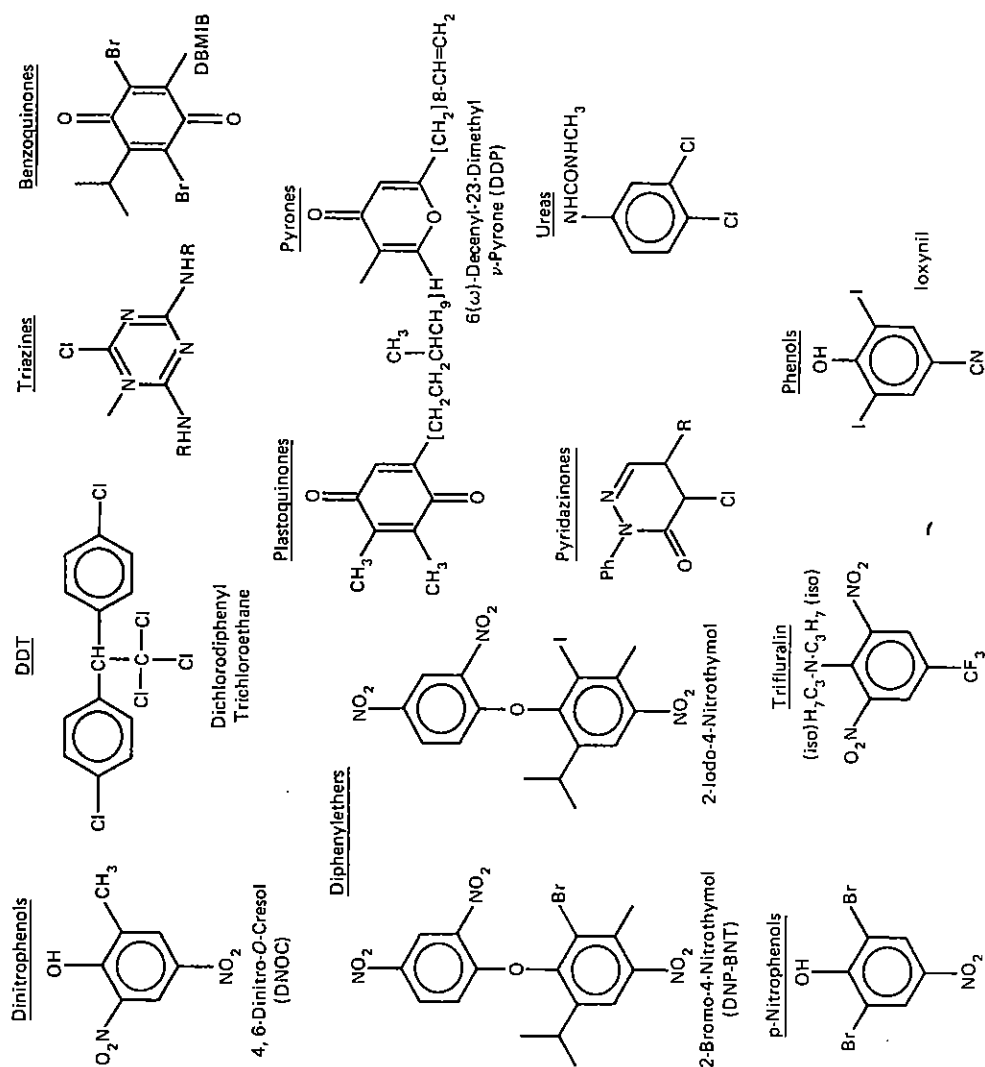


Fig. 4. Structures of important pesticides.

thesis and transpiration. Transpiration and photosynthesis declined by 9 and 8%, respectively, 24 hr after plants were treated with methomyl. One wk after treatment overall photosynthesis decreased to 20% of the normal rate. Methyl parathion also induced an initial 17% reduction in transpiration and 10% reduction in photosynthesis, which decreased to 27% and 18% reductions, respectively, after one wk. Methyl parathion amongst all had greatest effect on photosynthesis. Further, they noticed a steep reduction in lettuce yield following the application of these pesticides, which suggests the existence of a "pesticide threshold" in the plants. However, Sances *et al.* (1981) did not elucidate the functional mechanism of any of the given pesticides on photosynthesis.

Apart from the interference of pesticides with photochemical reactions, certain venomous substances, which are released into plants during insect feeding are also found to inhibit photosynthesis. The most recent report about such venomous substances is relating to melittin, a polypeptide component of bee-venom, devoid of enzymatic activity (Bishop and Kenrick 1980). Melittin consists of 26 amino acids of which the 20 N-terminal residues are hydrophobic and six C-terminal residues are basic. Melittin acts as an uncoupler of photophosphorylation and abolishes 518 nm light-induced absorbance changes. At higher concentrations (30 to 50 μ M), melittin abolishes both the light-induced photo-oxidation of Cyt f and partially inhibits other reactions of photosynthetic electron transfer, without causing lysis of the membrane. The uncoupled photo-reduction of MV from water (which is an index of PSI + PSII activity) is inhibited greater than 95% by 30 μ M melittin, while PSI activity (DCPIP/Ascorbate supported MV reduction) is inhibited over 85%. However, the inhibition of both the reactions can be relieved by carrier amounts of plastocyanin. Bishop and Kenrick (1980) reported that the inhibition of electron flow between two photosystems occurs mainly at the plastocyanin site. According to Bishop and Kenrick (1980), the concentration of melittin (5 μ M), which causes uncoupling and abolishes the 518nm absorbance changes, represents about one molecule of melittin/15 molecules of chloroplast polar lipid, while that totally inhibits MV reduction about one molecule of melittin/two molecules of polar lipid.

The major outcomes of these studies suggests that most of the compounds or a few of them at least in their course of action uncouple photosynthetic electron flow from phosphorylation and consequently no ATP formation would occur. Some of them like nitrofen (a diphenyl ether) and dinoseb (an alkylated dinitrophenol) do act as energy transfer inhibitors, rendering dissociation of ATP into ADP + iP (Younis and Mohanty 1980). Further the role of several herbicides or pesticides in the development of plastids is of much intrigue to scientists today. The effects of such uncoupling, resulting in no ATP formation, might be in any manner on the quality of the yields of the crop as observed by Sances *et al.* (1981) in respect to lettuce yield.

IV. Role of antibiotics

McCarty *et al.* (1965) had documented that 3.5 μg of Dio-9 (an antibiotic)/ml inhibited phosphorylation by 90% and ferricyanide reduction by 67%. They concluded that only electron transport which is tightly coupled to ATP formation is inhibited by Dio-9 and it was thus coined as "energy transfer" inhibitor.

Bishop (1973) and Nolan and Bishop (1975 and 1978) described the inhibition of photosynthetic electron flow by the polyene antibiotics such as filipin and amphotericin B. Nolan and Bishop (1975) showed that PSI activity is inhibited by the treatment of chloroplasts with 0.5M amphotericin B for 60 min at 25°C. Inhibition of amphotericin B is attributed to the release of plastocyanin from its site of action in the chloroplast membrane. The release is accompanied by a shift in the pH curve for FeCN photoreduction from water. Delayed light emission from PSII is not destroyed by amphotericin B treatment, indicating that PSII is not damaged in chloroplasts treated with amphotericin B, but they are unable to photo-oxidize PC, thus localizing the site of inhibition at PC. Nolan and Bishop (1978) reported that the inhibition of amphotericin B in maize could be due to the presence of sterols and due to the formation of pores in the thylakoid membranes. Amphotericin B is extensively used as antifungal antibiotic, also (Bajaj and Ghosh 1975). Earlier, Bishop (1973) reported that filipin, another polyene antibiotic, inhibits the activities of both PSII and PSI unlike amphotericin B which inhibits only PSI reactions. Maximum inhibition of activity was observed at about 0.4mM in maize mesophyll chloroplasts and 1.0mM in pea chloroplasts. The inhibition of PSI activity of both maize mesophyll and pea chloroplasts caused by filipin could be overcome by the addition of soluble electron-transfer protein PC. The inhibition of photochemical activity caused by filipin may be a secondary effect resulting from a change in membrane conformation induced by the antibiotic.

Treatment of isolated chloroplasts with valinomycin above $10^{-7}M$ inhibits both photophosphorylation and millisecond delayed light emission with maximum inhibition occurring at $10^{-5}M$ (Telfer and Barber 1974). The effects of valinomycin occur in the absence of added K^+ although the presence of K^+ does facilitate its action. Telfer and Barber (1974) opined that high concentration ranges of valinomycin not only induced changes in K^+ permeability but also in H^+ permeability of the thylakoid membranes and in doing so uncouple photophosphorylation as an energy transfer inhibitor. Voegeli *et al.* (1977) also reported that valinomycin inhibits the coupled portion of whole chain noncyclic electron transport and phosphorylation and also inhibits the menadione-dependent cyclic phosphorylation. According to Voegeli *et al.* (1977), valinomycin inhibition does not require addition of K^+ to the media. They observed that electron transport phosphorylation in various PSI and PSII partial reactions not involving PQ, using other cyclic-cofactors, that are not dependent on plastoquinone, were relatively insensitive to valinomycin. Thus the site of action of valinomycin is believed to be close to PQ.

Antimycin A, a macrolactone antibiotic produced by several species of streptomycetes, was extensively used as an effective antifungal antibiotic in horticulture and agriculture. In 1969 Gromet-Elhanan first reported that antimycin A had affected the cyclic photophosphorylation driven by PSI. Earlier Tagawa *et al.* (1963) documented that ferredoxin catalyzed cyclic photophosphorylation is the only cyclic system, which is antimycin A-sensitive. Izawa *et al.* (1967) opined that antimycin sensitivity of noncyclic electron flow would be apparent only under certain conditions like low light intensities, but Bohme *et al.* (1971) observed that Hill reactions with NADP is not antimycin sensitive up to a concentration of $10^{-5}M$, whereas cyclic photophosphorylation with ferredoxin is completely inhibited at $10^{-6}M$ of antimycin. Further, they reported that the slight decrease of coupled ATP formation in the NADP reduction above $5 \times 10^{-6}M$ of antimycin is due to an uncoupling. However, phenazine methosulphate (PMS) catalyzed cyclic photophosphorylation is not sensitive to antimycin up to a concentration of $5 \times 10^{-6}M$. In the presence of $10^{-5}M$ DBMIB, however, PMS-catalysed cyclic photophosphorylation does become antimycin sensitive. Bohme *et al.* (1971) noticed that this inhibition by antimycin in the presence of DBMIB ($10^{-5}M$) is incomplete and in contrast to the cyclic photophosphorylation reported by Tagawa *et al.* (1963) and Izawa *et al.* (1967). Antimycin concentration beyond $5 \times 10^{-6}M$ has no effect on ATP formation, since the decrease in ATP formation is due to uncoupling but not due to inhibition of electron transport.

Several other antibiotics like chloramphenicol and cycloheximide were used as potential antifungal antibiotics in agriculture. Their role in relation to the development of plastids is being studied (Nielson 1975). Nielsen (1975) studied the greening of barley leaves in the presence of sufficient chloramphenicol and cycloheximide. Cycloheximide was inhibitory to the chlorophyll synthesis at concentrations less than $10\mu g/ml$, while a 10-to-20-fold higher concentration of chloramphenicol was required to achieve the same inhibition. About 50% inhibition of chlorophyll synthesis was brought about at $1.4\mu M$ cycloheximide and $21\mu M$ chloramphenicol, respectively.

In short, the principles involved in the mode of action of these pesticides and antibiotics are just akin to that of cations but with a difference in the degree of severity, site of action, and mechanism. In other words, pesticides and antibiotics do cause (1) inhibition of electron flow (*i.e.*, rise in the level of fluorescence) or uncoupling from photophosphorylation (*i.e.*, stimulate electron flow devoid of ATP formation), and (2) conformational changes, as found applicable in respect of cations and salts. Additionally, pesticides and antibiotics inhibit energy transfer, also, rendering the dark phase of photosynthesis ineffective. Thus, a commonness in the mode of action could be derived.

After all, the problem of using many of the salts, pesticides, and antibiotics is supposedly manifold in relation to the soil and environmental conditions. For, at therapeutic level, many of these are phytotoxic and some get converted into metabolite, also (Bajaj and Ghosh 1975). There is a growing concern about the extensive application of these chemicals in agriculture in view of their toxicity

and possible carcinogenicity. It is hoped that the warning of the World Health Organization about the risk in using such chemicals would have some impact (Manten 1963). The fact that the dosage of each pesticide is specific and recommended is not sufficient to conclude that no hindrances occur in the vital metabolic activities of plants. Undoubtedly recommended dosages guarantee life to the plant but not the quality of the yield of the crop. It is, therefore, an ardent hope of the author that research on the development of pesticides would be directed towards analysing the effects of these pesticides on the quality of the crop.

Summary

A number of inorganic and organic chemicals which constitute a wide range of pesticides have been found to interfere with metabolic pathways of plants in general and with photosynthesis (a food processing mechanism of plants) in particular. Also, a number of chemicals which are present in soil due to various reasons have been found to accumulate in toxic proportions, though they are of nutritional value to plants at specific concentrations. Evidence has been presented here to show that a number of cations, salts, pesticides, and antibiotics have affected the chloroplast structure and function, rendering the mechanism of photosynthesis ineffective. The probable ways by which cations, salts, pesticides, and antibiotics could affect the photosynthetic mechanism are treated. It is concluded that almost all of those mentioned above could affect the photosynthetic mechanism either by inhibition of electron flow (resulting in either rise or decrease in the level of fluorescence from steady state fluorescence) or by uncoupling from photophosphorylation (resulting in no ATP formation). Further, they are found to cause conformational changes either by binding themselves to thylakoid membranes or releasing functional electron carriers from their site of action. Some are even found to solubilize lipids and some are also found to inhibit energy transfer. The entire research done in this direction had been reviewed here. Further, the need to initiate studies on the changes that occur in seed contents and are attributable to the effects of pesticides on photosynthesis have been emphasized here. However, very little work has been done in this direction and to arrive at any major conclusions in haste would be uncalled for.

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